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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: GATEY *et al.*

Serial No.: 09/401,839 Group Art Unit: 1646

Filed: September 22, 1999 Examiner: Mertz, Prima Maria

For: PURIFICATION AND CHARACTERIZATION OF CYTOTOXIC LYMPHOCYTE MATURATION FACTOR AND MONOCLONAL ANTIBODIES THERETO

(New) Attorney Docket No.: 11126-004

DECLARATION OF DR. WILLIAM R. BENJAMIN

Assistant Commissioner for Patents
Box AF
Washington, D.C. 20231

Sir:

I, William R. Benjamin, Ph.D., do declare that:

1. I am currently Vice President, Research Operations at Protein Design Labs, Inc., Fremont, CA, exclusive licensee of the captioned patent application. Prior to my position at Protein Design Labs, I was employed by Hoffmann-La Roche Inc. ("Roche"), assignee of the captioned patent application.

2. I have extensive experience in molecular biology and immunology. As a result of my experience, I am familiar with techniques utilized by the skilled artisan for generating monoclonal and polyclonal antibodies. For example, I am familiar with the standard techniques that would have been utilized by one of skill in the art during the 1988-1989 time frame.

3. My primary research interests relate to the discovery and evaluation of therapeutics for use in autoimmune and inflammatory diseases, infectious diseases and cancer. A copy of my curriculum vitae is attached hereto as Exhibit I.

4. I hold a Ph.D. in Medical Microbiology and Immunology, which I received in 1980 from the University of South Florida, College of Medicine. From 1981 to 1982 I was a postdoctoral fellow at the National Institute of Dental Research of the National Institutes of Health, Bethesda, MD.

5. I joined Roche, Nutley, NJ, in 1982 as a Senior Scientist in the Immunopharmacology Department. From 1985 to 1988 I was a Research Investigator in the department. I became a Research Leader and Head in 1988, and a Director of the department in 1989. In 1993 I became a Senior Director of the Inflammation and Autoimmune Diseases Research Department, and was named a Vice President of this department in 1995.

6. As pointed out above, in 1997 I joined Protein Design Labs, Inc., Fremont, CA, as Vice President of Drug Discovery.

7. As summarized in my attached curriculum vitae, I have authored or co-authored numerous scientific publications.

8. I have read and am familiar with United States Patent No. 5,811,523 ("'523 patent"), United States Patent No. 6,300,478 ("'478 patent"), the captioned application Serial No. 401,839 ("'839 application"), and the Office Action, mailed February 22, 2002, in connection with the '839 application. I have been advised that the original specification of application Serial No. 10/267,565, filed October 8, 2002 ("'565 application") is identical to that of the '839 application. I have also read and am familiar with United States patent application Serial No. 269,945, filed November 10, 1988 ("'945 application"), to which the '523 and '478 patents claim priority, and United States patent application Serial

No. 307,817, filed February 7, 1989 ("'817 application"), to which the '523 and '478 patents claim priority as continuations-in-part of the '945 application. In addition, I have read and am familiar with the scientific publications of Chizzonite *et al.*, a copy of which is attached hereto as Exhibit 2,[1] Stern *et al.*, a copy of which is attached hereto as Exhibit 3[2] and D'Andrea *et al.*, a copy of which is attached hereto as Exhibit 4.[3]

9. I understand that the subject matter claimed in the '523 and '478 patents and the '839 patent application relates to antibodies against Natural Killer Stimulatory ("NKSF") cytokine and Cytotoxic Lymphocyte Maturation Factor ("CLMF") cytokine, respectively. Each of NKSF and CLMF are heterodimers of two subunits, one approximately 30-35 kilodaltons ("kD") and one of approximately 40 kD, as measured under reducing conditions by sodium dodecyl sulfate polyacrylamide gel electrophoresis ("SDS PAGE"). Analyses of NKSF and CLMF have revealed that the two cytokines are the same, except for minor amino acid differences in one of the two subunits of the cytokine. The term "IL-12" has now become the preferred designation for this cytokine.

10. IL-12 (that is, NKSF and CLMF) exerts multiple effects on certain immune cells, such as T lymphocytes and natural killer cells. For example, IL-12 can induce synthesis of gamma interferon *in vitro* in human peripheral blood lymphocytes ("PBLs"), and can also induce proliferation of phytohemagglutinin ("PHA")-activated PBLs. IL-12 can

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[1] Chizzonite *et al.*, 1991, "IL-12: Monoclonal Antibodies Specific for the 40 kDa Subunit Block Receptor Binding and Biologic Activity of Activated Human Lymphoblasts," J. Immunol. 147: 1548-1556 ("Exhibit 2").

[2] Stern *et al.*, "Purification to Homogeneity and Partial Characterization of Cytotoxic Maturation Factor from Human B-lymphoblastoid Cells," Proc. Natl. Acad. Sci. USA 87: 6808-6812 ("Exhibit 3").

[3] D'Andrea *et al.*, 1992, "Production of Natural Killer Cell Stimulatory Factor (Interleukin 12) by Peripheral Blood Mononuclear Cells," J. Exp. Med. 176: 1387-1398 ("Exhibit 4").

also induce proliferation in an IL-12 dependent T cell growth assay. Moreover, CLMF is capable of binding to phytohemagglutinin activated peripheral blood lymphoblasts as determined in a CLMF receptor binding assay. As such, these biological activities are inherent properties of NKSF and CLMF. Neither subunit alone exhibits such biological activities.

11. Each of the biological activities of IL-12 is mediated by binding the IL-12 receptor. As such, antibodies that specifically react with IL-12 and that block one IL-12 biological activity would block all IL-12 biological activities.

12. With respect to claims 1-5 of the '523 patent and claims 1 and 2 of the '478 patent, I note that the recited antibody is one that specifically reacts, with an antigen that is "substantially free from association with other proteinaceous materials." The purity of an antigen, *e.g.*, an IL-12 antigen, with which an antibody specifically reacts is not relevant to identifying the antibody. That is, an antibody that specifically reacts with an antigen would do so whether the antigen is substantially free from association with other proteinaceous materials or is present in mixture with proteinaceous materials.

13. The '839 and '565 applications disclose, for example, several antibodies that specifically react with IL-12. Some of the disclosed antibodies are inhibitory, that is, block a biological activity of IL-12, and some are non-inhibitory. See, for example, '839 and '565 applications, p. 75, ll. 19-28. The '839 and '565 applications teach uses for blocking antibodies that specifically react with IL-12. For example, such blocking antibodies can be useful in lowering the concentration of biologically active IL-12 so as to achieve a selective blockade of proliferation and activation of cytotoxic T cells, such as in transplantation. See '839 and '565 applications, p. 7, ll. 2-4. Antibodies capable of blocking (or neutralizing) the biological activity of IL-12 are useful in ways that non-blocking antibodies are not useful because non-blocking antibodies cannot eliminate the biological

activity of IL-12. It is the blocking antibodies that are clearly useful, for example, to lower the concentration of biologically active IL-12 so as to achieve the selective blockade of proliferation and activation of cytotoxic T cells, *such as in transplantation. See* the captioned application, p. 7, ll. 2-4. Non-blocking antibodies would not be expected to be useful for that therapeutic application in transplantation.

14. Chizzonite *et al.* (Exhibit 2) reports on monoclonal and polyclonal antibodies directed against IL-12. The antibodies disclosed were generated using purified or partially purified IL-12[4] heterodimer. The results presented in Chizzonite *et al.* demonstrate that using purified NKSF resulted only in the generating of antibodies which specifically bind the 40 kD subunit (Chizzonite *et al.*, p. 1554, left column). That is, no antibodies were produced which specifically react with the 30-35 kD subunit. Chizzonite *et al.* also reports that, apparently, antibodies against the 30-35 kD subunit arise "only after multiple immunizations," as opposed to antibodies against the 40 kD subunit, which arise very rapidly (Chizzonite *et al.*, p. 1555, left column).

15. The techniques required by Chizzonite *et al.* to generate antibodies against the 30-35 kD subunit are not standard techniques that would have been utilized by one of skill in the art. Rather, using standard techniques, one skilled in the art, upon observing that immunized animals produced a sufficient titer of polyclonal antibodies that specifically react with IL-12, would have terminated the immunization schedule and sacrificed the animal. Thus, using standard techniques, further multiple immunizations would not have been done and antibodies specific for the 30-35 kD subunit would not have been made.

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[4] Chizzonite *et al.* refers to the cytokine as NKSF.

16. Conditions that stimulate production of biologically active NKSF heterodimer also induce production of a large excess free 40 kD NKSF subunit. See, *e.g.*, Chizzonite *et al.*, p. 1555, paragraph bridging left and right columns; and D'Andrea *et al.* (Exhibit 4), Figs. 4A-4B, p. 1392, and accompanying text. The 40 kD *subunit* alone, however, exhibits no biological activity. See, *e.g.*, Chizzonite *et al.*, Table III, p. 1553; and D'Andrea *et al.*, Figs. 4A-4B, p. 1392, and accompanying text. Thus, a successful antibody-based diagnostic use would require employing an antibody that can distinguish between the 70 kD NKSF heterodimer and the free 40 kD NKSF subunit.

17. The '945 application discloses a method that purports to partially purify IL-12 (see, for example, '945 application, p. 21, l. 12, to p. 24, l. 20). Note, however, that the free 40 kD subunit is present in excess in biological samples, and is also present in the partially purified IL-12 preparation described in the '945 application. Any such partially purified IL-12 preparation containing excess free 40 kD subunit, if used to elicit antibodies, would result in antibody preparations against both the free 40 kD subunit and IL-12. Such antibody preparations would co-purify the free 40 kD subunit and IL-12 and could not be used to selectively purify IL-12. Moreover, such resulting mixed preparations of IL-12 and free 40 kD subunit would not provide a suitable material for therapeutic applications.

18. The '945 application also discloses six peptides, each ranging in size from five to eight amino acid residues in length (see '945 application, p. 3, ll. 16-20; p. 11, ll. 12-17; and p. 27, ll. 5-10; the six sequences on each page are identical), then thought to be part of the amino acid sequence of IL-12. Three of those peptides are now known (but were not then known) to be found within the 30-35 kD subunit. See the three sequences at p. 11, ll. 14, 16 and 17 of the '945 application. Today it is clear from the amino acid sequence of Figs. 2A-2C of the '523 patent (col. 6, ll. 13-17) that these sequences correspond to the underlined amino acid residues 180-184, 246-252 and 81-88, respectively. In addition, it is

now known (but was not then known) that the remaining sequences at p. 11, ll. 12, 13 and 15 of the '945 application are found within the 40 kD subunit. See the '523 patent, Figs. 1A-1D, which shows that these sequences correspond to amino acids 75-79, 219-224 (with a mistake at 222) and 23-27 of the 40 kD subunit.

19. It would have been difficult and uncertain as to whether one could elicit antibodies to a protein containing any of such peptides, even if the peptide were to be conjugated to a carrier protein. The quality and specificity of such antibodies would also be doubtful. Rather, a peptide of five to eight amino acids, generally, a peptide of at least approximately ten amino acids, would be used in generating antibodies. Thus, it would have been difficult and uncertain as to whether one could elicit antibodies specific for IL-12 using those peptides of the 30-35 kD subunit disclosed in the '945 application.

20. The '817 application discloses an amino acid sequence that allegedly corresponds to the amino terminal sequence of the 30-35 kD subunit. See '817 application, p. 5, *l. 9.* A comparison of this sequence (top row) with the sequence now known to be present in the 30-35 kD subunit (bottom row) is shown below (amino acid residue positions in the mature sequence are indicated at the top):

2	3	4	5	6	7	8	9	10	11	12	13	14
Asn	Leu	Pro	Val	Ala	**Pro**	Pro	Asp	Pro	**[Ser or Thr]**	Met	Phe	Pro
Asn	Leu	Pro	Val	Ala	**Thr**	Pro	Asp	Pro	**Gly**	Met	Phe	Pro

21. It is readily apparent that there were two mistakes in this amino acid sequence of the '817 application, at positions 7 and 11. The longest correct sequence within it is five residues, which is unlikely to elicit antibodies that specifically react with the 30-35 kD subunit, even if it were conjugated to a carrier protein. Moreover, one skilled in the art would have considered it even less likely to elicit antibodies that specifically react with the 30-35 kD subunit using the entire 13-amino acid peptide of the '817 application, even if it

were conjugated to a carrier protein because the incorrect sequence of the 13-amino acid peptide significantly increases the possibility that antibodies raised against a peptide having that sequence would cross-react with other proteins. Specifically, if antibodies were raised against the 13-amino acid peptide that also bound to the correct sequence of the 30-35 kD subunit, this would confirm that they lacked specificity for the 30-35 kD subunit. Thus, at best, the antibody preparations that could be made using the disclosure provided in the '817 application would be ones that react with the free 40 kD subunit and with the 40 kD subunit present in IL-12.

22. In the Stern *et al.* publication (Exhibit 3), a monoclonal anti-CLMF antibody is described that is referred to as "7B2." See Stern *et al.*, page 6809. One of the authors of the Stern *et al.* publication has indicated to me that the 7B2 antibody of Stern *et al.* is the same antibody designated "7B2" in the '839 and '565 applications. Further, based upon my knowledge of the amino acid sequence of the 40 kD subunit of the CLMF of the '839 application, the Stern *et al.* publication and the two NKSF proteins of the '523 and '478 patents, in my opinion monoclonal antibody 7B2 would specifically react with the 40 kD subunit of all three proteins and would specifically react with such CLMF and two NKSF proteins. In addition, and based upon the data in the captioned application, in my opinion monoclonal antibody 7B2 blocks the biological activities of such CLMF and two NKSF proteins, which would include their gamma interferon production inducing activity.

23. I note that claim 5 of the '523 patent recites a *human* antibody that reacts with NKSF (*i.e.*, IL-12). However, methods for how to make a *human* antibody directed against a *human* antigen without having to resort to undue experimentation were beyond the state of the art at the time either the '817 or the '945 applications were filed. That is, significantly more than standard techniques would have been required to generate such

human antibodies since the first publications demonstrating human phage display were in 1990.

I further declare that all statements made in this Declaration are of my own knowledge and are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 1/17/03

William R. Benjamin, Ph.D.

Attachments:

Exhibit 1: Curriculum Vitae of William R. Benjamin, Ph.D.

Exhibit 2: Chizzonite *et al.*, 1991, J. Immunol. 147: 1548-1556 ("Chizzonite *et al.*")

Exhibit 3: Stern *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87: 6808-6812 ("Stern *et al.*")

Exhibit 4: D'Andrea *et al.*, 1992, J. Exp. Med. 176: 1387-1398 ("D'Andrea *et al.*")